L.F., Ondrechen, M.J., Wilson, I.A., et al. (2008). Immunity *28*, 774–786.

Scharf, L., Li, N.-S., Hawk, A.J., Garzón, D., Zhang, T., Fox, L.M., Kazen, A.R., Shah, S., Haddadian, E.J., Gumperz, J.E., et al. (2010). Immunity *33*, this issue, 853–862.

Shamshiev, A., Gober, H.J., Donda, A., Mazorra, Z., Mori, L., and De Libero, G. (2002). J. Exp. Med. *195*, 1013–1021.

Spada, F.M., Grant, E.P., Peters, P.J., Sugita, M., Melián, A., Leslie, D.S., Lee, H.K., van Donselaar, E., Hanson, D.A., Krensky, A.M., et al. (2000). J. Exp. Med. *191*, 937–948.

Van Rhijn, I., Young, D.C., De Jong, A., Vazquez, J., Cheng, T.Y., Talekar, R., Barral, D.C., León, L., Brenner, M.B., et al. (2009). J. Exp. Med. *206*, 1409–1422.

CuRTAiling Interferon Regulatory Factor Signaling with the E3 Ligase RAUL

Suzanne Paz[1] and John Hiscott[1,2,*]
[1]Lady Davis Institute, Jewish General Hospital, McGill University, Montreal, H3T 1E2, Canada
[2]Vaccine & Gene Therapy Institute of Florida, Port St. Lucie, FL 34987, USA
*Correspondence: john.hiscott@mcgill.ca
DOI 10.1016/j.immuni.2010.12.006

Interferon regulatory factor 3 (IRF3) and IRF7 modulate the transcription of type I interferon. In this issue of *Immunity*, Yu and Hayward (2010) identify RAUL, the bona fide ubiquitin ligase that regulates turnover of IRF3 and IRF7.

Host protection against viral infection is orchestrated through specific pathogen sensing, complex signaling pathways, and a diversity of protective responses. The initial efforts of the infected host cell are to limit virus replication by engaging a broad spectrum of innate antiviral mechanisms while signaling the initiation of adaptive immunity. Microbial pathogens express defined pattern-associated molecular patterns (PAMPs) that are detected by pattern-recognition receptors (PRRs). Thus far, three PRR families have been identified: the Toll-like receptors (TLRs), the cytoplasmic retinoic acid inducible gene (RIG-I)-like receptors (RLRs), and the nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) (Akira et al., 2006; Takeuchi and Akira, 2010). Engagement of a PRR will activate a specific signaling cascade culminating in the expression of downstream target genes such as type I interferon (IFN), inflammatory mediators, and host restriction factors. Common to most PRR signaling events is the activation of interferon regulatory factors—IRF3 or IRF7, critical transcriptional activators of type I IFN gene activation. IRF3 and/or IRF7 are phosphorylated by the noncanonical IκB -related kinases (IKK) TANK-binding kinase (TBK1) and IKKε at key serine residues in the C terminus of the protein after TLR3, TLR4, or RIG-I stimulation.

The active state of these transcription factors is tightly regulated by posttranslational modifications such as phosphorylation, ubiquitination, and the addition of interferon stimulated gene 15 (ISG15), also known as ISGylation. Recognition that protein ubiquitination functions as a major regulator of signal transduction has refocused the search for negative regulators to the identification of E3 ligases with the potential to modulate IRF activity and the IFN response. As with the identification of novel kinases, significant effort has been directed to the discovery of E3 ligases because they define the specificity and complexity that is characteristic of the ubiquitination process. In this issue, Yu and Hayward (2010) identify RAUL as the bona fide E3 ligase that conjugates K48-dependent Ub chains to IRF3 and IRF7, leading to proteasome mediated degradation.

Ubiquitination, like phosphorylation, is a reversible process controlled by a limited number of deubiquitinases that specifically cleave ubiquitin chains (Ribet and Cossart, 2010). Ubiquitin (Ub) is a small peptide of 76 amino acids that is highly conserved in all eukaryotic cells. Ub becomes conjugated to a target protein via formation of an isopeptide bond between its C terminus (G76) and the ε-amino group of an acceptor Ub lysine residue (most commonly studied are K48 or K63)—or directly to the amino terminus of the target protein. Alternatively, head-to-tail linked Ub moieties have been described recently, as part of the novel process of linear ubiquitination (Clague and Urbe, 2010). This highly organized posttranslational modification requires the sequential action of three enzymes: E1-E2-E3. A ubiquitin-activating enzyme (E1) forms a thiol ester with the carboxyl group of Ub, thereby activating it for nucleophilic attack. A conjugating enzyme (E2) transiently carries the activated Ub molecule as a thiol ester, and the E3 ligase transfers the activated Ub from the E2 to the substrate.

A common strategy of many viruses is to target key antiviral signaling proteins for proteasomal degradation by using either viral or host ubiquitin ligases (Viswanathan et al., 2010). Pathogens hijack certain posttranslational modifications to preferentially target specific host pathways to promote their replication and propagation and to escape from the immune system (Ribet and Cossart, 2010) . Viral proteins that usurp the ubiquitin-proteasome pathway include the E6- associated protein (E6-AP) of human

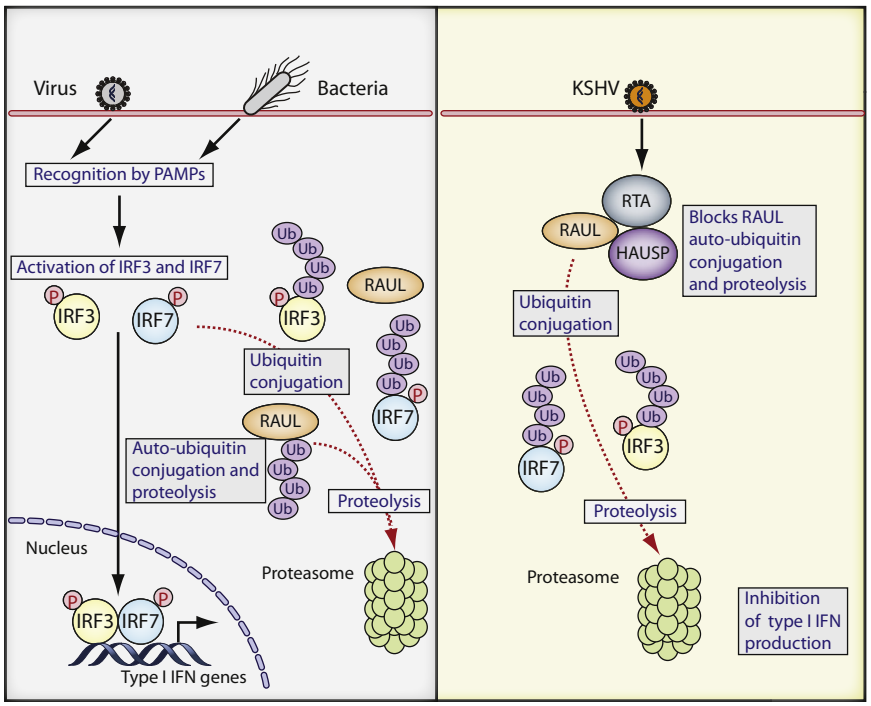

Figure 1. Ubiquitination of IRFs by RAUL-RTA-HAUSP
Upon recognition of PAMPs by their respective PRR, signal complex formation leads to IRF3 and IRF7 phosphorylation, activation, and type I IFN gene activation. Active IRF3 and IRF7 undergo proteasomal degradation, controlled by RAUL whose ligase activity is mediated by self-ubiquitination and proteolysis. In the context of KSHV infection, viral E3 ligase RTA will hijack RAUL and recruit deubiquitin enzyme HAUSP. HAUSP is responsible for deubiquitinating RAUL, leading to a stable and more active RAUL which in turn will more readily promote IRF3-IRF7 proteasomal degradation.

papilloma virus, infected cell protein 0 (ICP0) of herpes simplex virus type 1, E4orf6 and E1B55K of the adenovirus family, viral infectivity factor (VIF) of lentiviral viruses such as human immunodeficiency virus (HIV), the V protein of rubulavirus, and replication and transcription activator (RTA) of Kaposi's sarcoma-associated herpesvirus (KSHV) (Viswanathan et al., 2010). Relevant to the present study is the role of RTA, also known as open reading frame 50 (Orf50), from KSHV. RTA is a DNA-binding nuclear transcription factor acting throughout the virus replication cycle. Yu and Hayward previously demonstrated that RTA could bind IRF7 and induce polyubiquitination and proteasome-mediated degradation, via the action of the E2 ligase UbcH5α (Yu et al., 2005). They briefly mention that RTA also affected IRF3 stability without detailing their findings, but demonstrated that RTA controlled its own polyubiquitination and stability.

In this issue, Yu and Hayward (2010) have extended their previous studies and provide compelling evidence that the RTA-associated ubiquitin ligase (RAUL) directly catalyzes K48-linked polyubiquitination of IRF3 and IRF7, tagging them for proteasomal degradation under both homeostatic and pathologic conditions (Figure 1). An insightful yeast two-hybrid screen first identified KSHV immediate-early lytic cycle protein RTA as having the potential binding partners of RAUL and IRF7. Beginning with an exploration of the target specificity of RAUL, the authors have shown that the continuous activity of RAUL serves to maintain homeostatic basal amounts of IRF3 and IRF7. RAUL was capable of binding to IRF3 and IRF7 independently of their phosphorylation status, making it a good candidate for controlling basal protein expression of IRF3 and IRF7. Both in vivo and in vitro, RAUL directly conjugated K48-polyubiquitin chains to IRF3 and IRF7, thus leading to their proteasomal degradation. In this regard, the authors utilized various approaches to validate their findings, from silencing RAUL to using mutated forms of the E3 ligase in overexpression and/or endogenous systems. A cell-free assay demonstrated the ability of RAUL to directly ubiquitinate IRF3 and IRF7 via the E2 ligase UbcH5α. However, an important finding missing from their comprehensive ubiquitination studies was the identification of the key lysine residues of IRF3 and IRF7 targeted by RAUL. An unexplained aspect of their findings is the role of RAUL in maintaining the steady-state amounts of IRF3 and IRF7—a curious observation considering that IRF3 and IRF7 have notable differences in their stability profile. IRF7 has a half-life of ~30 min and contains a PEST (proline [P], glutamic acid [E], serine [S], and threonine [T]) domain in its N-terminus, indicative of an unstable protein, whereas IRF3 is a stable protein with a half-life of ~24 hr and does not possess a PEST domain. Can RAUL differentiate between IRF3 and IRF7 sequence or structure, thus preferentially promoting IRF7 instability over IRF3? It remains unclear how RAUL select its targets; determining this would reveal whether RAUL distinguishes between inactive versus phosphorylated IRF3 and IRF7.

The authors proceeded to investigate the antiviral properties of RAUL in KSHV and Sendai virus infection models. In both instances, the depletion of RAUL with siRNA silencing led to an increase in IFN-β production with a concomitant decrease in viral replication. Reciprocal results—decreased IFN production and augmented viral replication—were observed when RAUL was ectopically overexpressed, thus demonstrating a negative regulatory role for the E3 ligase RAUL in IFN production (Yu and Hayward, 2010).

Although it has been known for more than a decade that IRF3 is targeted for proteasomal degradation after virus infection (Lin et al., 1998), the E3 ubiquitin ligase and related signals responsible for its degradation have remained elusive. Previous studies have identified several putative cellular E3 ligases involved in the ubiquitination of IRF3, such as Ro52, Cull-1, RBCK1, and SOCS1 (Higgs and Jefferies, 2008), as well as the peptidyl-prolyl isomerase Pin1 (Saitoh et al., 2006). In the context of IRF7, RIP-1, TRAF6, RTA, and LMP1 were identified as E3 ligases, of cellular and viral origin, capable of regulating type I IFN by orchestrating IRF7 ubiquitination (Higgs and Jefferies, 2008). An important consideration in the present study is the fact that the

authors also investigated the previously identified E3 ligases RBCK1 and Ro52 and their involvement in the IRF3 ubiquitination. Their results demonstrated that these E3 ligases play a role in negative regulation of IRF3, but RAUL is the principal E3 ligase involved in degradation of IRF3 and IRF7 (Yu and Hayward, 2010).

The authors broadened their study of RAUL by revealing a role for viral RTA in the evasion of the host immune response. It is this link with KHSV pathogenesis that further distinguishes this study (Yu and Hayward, 2010). They demonstrated that RTA can hijack cellular RAUL and modulate the stability of RAUL by recruiting the deubiquitinating enzyme HAUSP. Silencing HAUSP led to an increase in RAUL stability by preventing self-ubiquitination and proteolysis. By taking control of HAUSP, RTA enhanced RAUL activity, leading to greater IRF3 and IRF7 proteasomal degradation. Considering that RTA itself possesses E3 ligase activity capable of targeting IRF3 and IRF7 for degradation (Yu et al., 2005), the question arises as to why KSHV goes through the trouble of hijacking RAUL and recruiting HAUSP, instead of simply using RTA to promote IRF3 and IRF7 degradation? The question may be more complex, considering that IRF3 is essential for induction of host restriction factors within the context of an ongoing IFN response. Redundantly targeting both IRF3 and IRF7 for degradation may be a safeguard in the context of virus replication and immune evasion. These anti-IRF countermeasures may also serve as a switch between cycles of lytic and latent KSHV infection. Perhaps the RTA-HAUSP-RAUL complex also assists KSHV to downregulate its viral IRF-like proteins that are induced during viral reactivation and thus favors a shift toward latent infection. Beyond the scope of the authors' contributions to the understanding of RAUL biology and IRF degradation, these findings are likely to provide insights into the pathogenesis of KSHV-associated disease.

REFERENCES

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Cell *124*, 783–801.

Clague, M.J., and Urbe, S. (2010). Cell *143*, 682–685.

Higgs, R., and Jefferies, C.A. (2008). Biochem. Soc. Trans. *36*, 453–458.

Lin, R., Heylbroeck, C., Pitha, P.M., and Hiscott, J. (1998). Mol. Cell. Biol. *18*, 2986–2996.

Ribet, D., and Cossart, P. (2010). Cell *143*, 694–702.

Saitoh, T., Tun-Kyi, A., Ryo, A., Yamamoto, M., Finn, G., Fujita, T., Akira, S., Yamamoto, N., Lu, K.P., and Yamaoka, S. (2006). Nat. Immunol. *7*, 598–605.

Takeuchi, O., and Akira, S. (2010). Cell *140*, 805–820.

Viswanathan, K., Fruh, K., and DeFilippis, V. (2010). Curr. Opin. Microbiol. *13*, 517–523.

Yu, Y., and Hayward, G.S. (2010). Immunity *33*, this issue, 863–877.

Yu, Y., Wang, S.E., and Hayward, G.S. (2005). Immunity *22*, 59–70.

Foxo1 and Foxo3 help Foxp3

Naganari Ohkura[1,2,*] and Shimon Sakaguchi[1,2]
[1]Department of Experimental Immunology, World Premier International Immunology Frontier Research Center, Osaka University, Suita 565-0871, Japan
[2]Department of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: nohkura@frontier.kyoto-u.ac.jp
DOI 10.1016/j.immuni.2010.12.004

In this issue of *Immunity*, Kerdiles et al. (2010) report that Foxo transcription factors are essential for the development and function of Foxp3-expressing regulatory T (Treg) cells via controlling the expression of genes associated with Treg cell function.

Foxo transcription factors belong to the Forkhead box family of transcription factors characterized by a conserved winged helix DNA binding domain. In mammals, the Foxo subfamily is comprised of four members, Foxo1, Foxo3, Foxo4, and Foxo6. Foxo1 and Foxo3 are the main isoforms expressed in the immune system. They are important regulators of cell cycle progression, apoptosis, glucose metabolism, and stress resistance via integrating information of the presence of nutrients, growth factors, and stress signals. Recent studies have shown that Foxo transcription factors are also associated with lymphocyte functions such as gene recombination, homing, and cytokine receptor expression. Although Foxo transcription factors appear to play important roles in a variety of biological processes, the functions of Foxo1 and Foxo3 in T cells still remain obscure. In this issue of *Immunity*, Kerdiles et al. (2010) investigate autoimmunity resulting from T cell-specific deletion of Foxo1 and additional deletion of Foxo3. They conclude that Foxo transcription factors are essential for specifying the program of T cell differentiation especially into regulatory T (Treg) cells expressing the transcription factor Foxp3 (Figure 1).

Foxo transcription factors can act as either transcriptional activators or repressors by forming different molecular complexes with different transcriptional modulators including β-catenin, STAT3, Runx3, Smad3, or Smad4. In addition, their function is tightly regulated by the upstream phosphoinositide 3-kinase (PI3K) and Akt pathway, which phosphorylates Foxo molecules and facilitates their nuclear export into the cytoplasm. After